American Journal of Medical Genetics 61:283–289 (1996)

Mitochondrial DNA Sequence Analysis of Four Alzheimer's and Parkinson's Disease Patients

Michael D. Brown, John M. Shoffner, Yoon L. Kim, Albert S. Jun, Brett H. Graham, Margaret F. Cabell, Daniel S. Gurley, and Douglas C. Wallace

Department of Genetics and Molecular Medicine, Emory University School of Medicine, Atlanta, Georgia

The mitochondrial DNA (mtDNA) sequence was determined on 3 patients with Alzheimer's disease (AD) exhibiting AD plus Parkinson's disease (PD) neuropathologic changes and one patient with PD. Patient mtDNA sequences were compared to the standard Cambridge sequence to identify base changes. In the first AD + PD patient, 2 of the 15 nucleotide substitutions may contribute to the neuropathology, a nucleotide pair (np) 4336 transition in the $tRNA^{Gln}$ gene found 7.4 times more frequently in patients than in controls, and a unique np 721 transition in the 12S rRNA gene which was not found in 70 other patients or 905 controls. In the second AD + PD patient, 27 nucleotide substitutions were detected, including an np 3397 transition in the ND1 gene which converts a conserved methionine to a valine. In the third AD + PD patient, 2 polymorphic base substitutions frequently found at increased frequency in Leber's hereditary optic neuropathy patients were observed, an np 4216 transition in ND1 and an np 13708 transition in the ND5 gene. For the PD patient, 2 novel variants were observed among 25 base substitutions, an np 1709 substitution in the 16S rRNA gene and an np 15851 missense mutation in the cytb gene. Further studies will be required to demonstrate a causal role for these base substitutions in neurodegenerative disease.

KEY WORDS: mitochondrial DNA sequence, Alzheimer's disease, Parkinson's disease, synergistic genetic interactions

INTRODUCTION

Alzheimer's disease (AD) and Parkinson's disease (PD) are late-onset, progressive, neurodegenerative diseases associated with dementia and movement disorders, respectively. Neuropathologically, AD is associated with cortical accumulation of senile plaques and neurofibrillary tangles while PD is associated with Lewy body accumulation. However, these genetically heterogeneous disorders can overlap neuropathologically and clinically. Extrapyramidal signs of PD have been observed in AD patients [Chui et al., 1985; Mayeux et al., 1985], and 20–40% of neuropathologically confirmed AD patient brains were found to have substantia nigra degeneration and Lewy body formation in the pigmented nuclei and nucleus basalis (AD + PD patients) [Ditter and Mirra, 1987]. Also, "diffuse Lewy body disease" has been noted in individuals with and without concurrent AD pathology [Byrne et al., 1989; Dickson et al., 1989; Crain and Mirra, 1990; Hansen et al., 1990; Perry et al., 1990]. Such overlap in these 2 late-onset, tissue-specific diseases may suggest common or related causal factors in a subset of patients.

Because the brain is highly dependent on mitochondrial ATP production via oxidative phosphorylation (OXPHOS), and because OXPHOS capacity declines naturally with age [Trounce et al., 1989; Cooper et al., 1992; Bowling et al., 1993; Boffoli et al., 1994], it has been hypothesized that some late-onset, progressive neurodegenerative diseases are the result of mitochondrial abnormalities [Wallace, 1992a,b]. Mitochondrial dysfunction has been reported in both AD and PD, with abnormal mitochondria containing paracrystalline inclusions observed in brain tissue of AD and AD + PD patients [Saraiva et al., 1985; Hansen et al., 1989]. In AD, mitochondrial respiratory complex IV enzyme defects have been reported for platelets [Parker et al., 1990; Parker et al., 1994a], fibroblasts [Peterson and Goldman, 1986], and brain [Parker et al., 1994b]. In PD, complex I defects as well as multiple OXPHOS enzyme deficiencies have been detected in various tissues [see Schapira, 1994 for review] such as brain [Mizuno et al., 1989; Schapira et al., 1989, 1990; Hattori et al., 1991; Mann et al., 1992], skeletal muscle [Shoffner et al., 1991; Bindoff et al., 1991; Nakagawa-Hattori et al., 1992; Cardellach et al., 1993; Blin et al., 1994], and

Received for publication May 11, 1995; revision received August 24, 1995.

Address reprint requests to Dr. Douglas C. Wallace, Department of Genetics and Molecular Medicine, Emory University School of Medicine, 403 Dental Building, 1462 Clifton Road, Atlanta, GA 30322.

platelets [Parker et al., 1989; Yoshino et al., 1991; Krige et al., 1992; Benecke et al., 1993].

The prevalence of mitochondrial OXPHOS dysfunction in AD, PD, and AD + PD patients, coupled with the genetic heterogeneity [Golbe, 1990; Johnson, 1991; Mullan et al., 1992; St. George-Hyslop et al., 1992; Van Broeckhoven et al., 1992; Strittmatter et al., 1993] and atypical inheritance patterns evident in these diseases, suggested that mitochondrial DNA (mtDNA) mutations may contribute to the expression of these diseases in at least some patients. Recently, we identified 4 novel mtDNA mutations in 71 Caucasian AD, PD, and AD + PD patients after restriction fragment length polymorphism (RFLP) and phylogenetic analysis [Shoffner et al., 1993]. A mutation at nucleotide pair (np) 4336 in the $tRNA^{Gln}$ gene was found in 9 patients. This mutation changed a moderately conserved nucleotide and was 7.4 times more prevalent in patients than in Caucasian controls. Further, this mutation defined a branch on the phylogenetic tree, upon which 9 of the 10 individuals had AD, PD, or AD + PD. The association of this mutation with AD was also demonstrated by Hutchin and Cortopassi [1995], who found the np 4336 mutation at a greater than 20-fold higher frequency in patients than in race and age-matched controls. A second mutation at np 3397 of the ND1 gene was found in 2 unrelated AD + PD patients, one of whom also harbored the np 4336 mutation. The np 3397 mutation converts a highly conserved methionine to a valine and was not found in 248 Caucasian controls. A third mutation was an insertion of approximately 5 cytosines between nps 956 and 965 in the 12S rRNA gene; the first example of an intragenic insertion and structural alteration of a human mtDNA rRNA gene. This mutation was found in a single AD + PD patient who also harbored the np 4336 mutation, but was not found in 699 control subjects including 119 Caucasians. A fourth mutation at np 3196 of the 16S rRNA gene was found in one AD patient and was heteroplasmic. It was also detected in one of 699 controls assayed, an Asian who was homoplasmic. Because the RFLP analysis only surveyed approximately 20% of the mtDNA sequence, it remained possible that other disease-associated mtDNA variants existed in these patients. Such variants could contribute to the phenotype through synergistic interaction with the observed RFLP variants, a pathogenetic mechanism which may influence the expression of another adult-onset neurodegenerative disease caused by mtDNA mutations, Leber's hereditary optic neuropathy [LHON; Johns and Berman, 1991; Brown et al., 1992a,b].

In order to fully investigate the role of mtDNA mutations in these late-onset neurodegenerative diseases, we determined the entire mtDNA sequence in 3 AD + PD and one PD patient. One of these patients harbored the np 4336 mutation and another harbored only the np 3397 mutation. No additional relevant variants were observed in the np 3397-positive mtDNA, while the np 4336-positive mtDNA harbored a unique 12S rRNA np 721 variant. The remaining AD + PD mtDNA harbored variants that are thought to contribute to LHON, and the PD mtDNA harbored 2 novel transitions, one in the 16S rRNA and the other in the cytb gene.

MATERIALS AND METHODS

Patient and Control Samples

The AD + PD mtDNA samples were obtained from autopsy brains showing the neuropathologic changes of AD + PD [Ditter and Mirra, 1987]. Caucasian AD + PD patients ADPD2, ADPD7, ADPD14 presented with AD phenotype, but with both senile plaques and neurofibrillary tangles plus Lewy bodies. Skeletal muscle mtDNA was obtained from Caucasian PD patient PD4 who was clinically identified and was responsive to levodopa. A subset of the 71 AD, PD, and AD + PD patients described by Shoffner et al. [1993], nearly all of whom were neuropathologically ascertained, were screened for certain additional mtDNA mutations found in sequencing the above 4 patients. Unaffected Caucasian control mtDNAs used in this study represent random, healthy Caucasians of various ages primarily from throughout the United States and Canada. These Caucasian controls therefore do not overrepresent any particular Caucasian subpopulation. Control mtDNA was extracted from the platelets or the buffy coat blood fraction.

MtDNA Sequence Analysis

Genomic DNA was isolated from brain (ADPD2, ADPD7, and ADPD 14) or skeletal muscle (PD4) as previously described [Corral-Debrinski et al., 1991]. DNA sequence analysis was accomplished by direct sequencing of asymmetrically polymerase chain reaction (PCR)-amplified mtDNA as described in Brown et al. [1992b]. Both *Taq* (Amplitaq, Perkin-Elmer/Cetus) DNA polymerase and T7 (Sequenase; United States Biochemical) DNA polymerase were used in dideoxyribonucleotide chain-termination sequencing reactions. The mitochondrial protein-coding, tRNA, and rRNA genes were sequenced, though the noncoding D-loop region (approximately 1,000 nucleotides) was not. Sequence ambiguities were resolved either by sequencing the opposite DNA strand, by restriction enzyme analysis, or by primer-mismatch PCR to create a diagnostic recognition site [Brown et al., 1992b].

Population Survey of mtDNA Variants

Patient and control populations were screened for mutations at nps 1189, 1709, 1811, 3197, and 13934 using mutation-specific restriction endonuclease digestion. Prior to digestion, a diagnostic recognition site was created by primer-mismatch PCR [Brown et al., 1992b]. Restriction enzyme digestion products were separated using 2.5% NuSieve plus 0.95 SeaKem agarose gels containing 1 × TBE Buffer and 1 μg/ml ethidium bromide.

For the np 1189 mutation, the forward PCR primer extended from np 1169 to 1188 (5′ to 3′) with a mismatched G at np 1186. This creates a *Rsa*I site when the np 1189 mutation is present. When paired with a reverse primer from np 1467 to np 1486 (3′ to 5′), the resulting 317 base pair (bp) PCR product was cleaved by *Rsa*I into 179 and 138 bp fragment for normal and 179, 118, and 20 bp fragments for mutant mtDNAs.

For the np 1709 mutation, the forward PCR primer extended from np 1682 to np 1708 (5′ to 3′) with a mismatched T at np 1705 and a mismatched G at np 1707. This creates a *Sfu*I site when the np 1709 mutation is present. When paired with a reverse primer from np 2060 to np 2079 (3′ to 5′), the resulting 397 by PCR product was cleaved by *Sfu*I into 370 and 27 bp fragments when the mutation was present.

For the np 1811 mutation, the forward PCR primer extended from np 1784 to np 1810 (5′ to 3′) with a mismatched C at np 1808. This creates a *Rma*I site in the PCR product when the np 1811 mutation is present. When paired with a reverse primer from np 2060 to 2079 (3′ to 5′), the resulting 295 bp PCR product was cleaved by *Rma*I into 214 and 81 bp fragments for normal and 214, 54, and 27 bp fragments for mutant mtDNAs.

For the np 3197 mutation, the forward PCR primer extended from np 3170 to np 3196 with a mismatched G at np 3191. This creates an *Esp*I site in the PCR product when the np 3197 mutation is present. When paired with a reverse primer from np 3351 to np 3370 (3′ to 5′), the resulting 200 bp PCR product was cleaved by *Esp*I into 173 and 27 bp fragments when the mutation was present.

For the np 13934 mutation, the forward primer extended from np 13907 to np 13933 (5′ to 3′) with a mismatched G at np 13931 and a mismatched T at np 13932. This creates a *Bst*1107 I site when the np 13934 mutation is present. When paired with a reverse primer from np 14128 to np 14147 (3′ to 5′), the resulting 240 bp PCR product was cleaved by *Bst*1107 I into 213 and 27 bp fragments when the mutation was present.

RESULTS

Three Caucasian AD + PD (ADPD2, ADPD7, and ADPD14) patients and one Caucasian PD (PD4) patient were chosen for complete mtDNA sequence analysis in an attempt to find pathogenic mtDNA mutations which contribute to AD and/or PD. Patients ADPD2, ADPD7, and ADPD14 were selected for sequence analysis based on a previous mtDNA RFLP study by Shoffner et al. [1993], which suggested an mtDNA etiological role for these patients.

In excess of 92% of all protein coding genes, 95% of the rRNA genes, and 96% of all tRNA genes were sequenced in the 4 patients. Sequence results were compared to the standard Cambridge mtDNA sequence to identify mutations [Anderson et al., 1981].

Patient ADPD7 harbored 15 mtDNA sequence differences relative to the Cambridge sequence (Table I). Twelve of these could be excluded because they were silent mutations, common polymorphisms, or errors in the Cambridge sequence. Of the remaining 3 sequence variants, one was the T to C transition at np 4336 in the $tRNA^{Gln}$ gene previously identified in AD, PD, and AD + PD patients by haplotype analysis [Shoffner et al., 1993]. The second was a T to C transition at np 721 in the 12S rRNA gene which alters a nonconserved rRNA nucleotide and creates a *Hinf*I restriction endonuclease site. Using the *Hinf*I test, the mutation was confirmed in ADPD7 but was not found in 70 other Caucasian AD, PD, and AD + PD patients. This variant was not detected in 106 Caucasian [Shoffner et al., 1993 and unpublished data, this laboratory], 659 Asian [Torroni et al., 1992, 1993a,b; Ballinger et al., 1992], or 140 African control mtDNAs [unpublished data, this laboratory], bringing the total number of controls to 905. The third variant was a G to A transition at np 13759 in the ND5 gene which changes the nonconserved amino acid 175 from an alanine to a threonine (Table II). This mutation has not been previously described in at least 36 published ND5 sequences [Wallace et al., 1988; Shoffner et al., 1990; Howell et al., 1991a,b; Johns and Berman, 1991; Brown et al., 1992a,b; Marzuki et al., 1991; Obayashi et al., 1992; Ozawa et al., 1991a,b; Mackey and Howell, 1992; Letrit et al., 1992; Prezant et al., 1993; Kobayashi et al., 1990; Howell et al., 1993]. However, the mutation does create a *Sfa*NI restriction endonuclease site at np 13759. Using this assay, the mutation was detected in one of 54 (1.9%) ADPD patients, ADPD7 being the single positive patient, and in one of 50 (2.0%) Caucasian controls. Since patient ADPD7 and the unaffected individual did not have similar mtDNA haplotypes, this mutation must have occurred twice independently.

Patient ADPD2 harbored 27 mtDNA mutations compared to the Cambridge sequence (Table I). Twenty-two of these variants were excluded because they were either silent mutations, common polymorphisms, or Cambridge sequencing errors. Of the remaining 5 mutations, 3 were missense mutations. The first was the previously identified A to G transition at np 3397 in the ND1 gene [Shoffner et al., 1993]. The other 2 missense mutations were an A to G transition at np 4732 of the ND2 gene and an A to G transition at np 13637 of the ND5 gene (Table II). The np 4732 mutation changed a nonconserved asparagine to a serine and created an *Rsa*I restriction endonuclease recognition site. This site gain was detected in a total of 2 of 74 (2.7%) AD, PD, or AD + PD patients [including ADPD2; Shoffner et al., 1993]; one of 106 (0.9%) Caucasian controls [Shoffner et al., 1993; unpublished data, this laboratory]; one of 45 (2.2%) Tibetan controls [Torroni et al., 1994]; and one of 153 (0.7%) Siberian controls [Torroni et al., 1993b]. The np 13637 mutation changes a nonconserved glutamine to an arginine and creates a *Taq*I restriction site. This variant was detected in 4 of 74 (5.4%) AD, PD, or AD + PD patients; 3 of 106 (2.8%) Caucasian controls [Shoffner et al., 1993; unpublished data, this laboratory]; and one of 485 (0.2%) American Indian control mtDNAs [Torroni et al., 1992, 1993a]. The 2 remaining variants occur in the 16S rRNA gene. One is a nonconserved C to T transition at np 1721 which creates an *Alu*I restriction site which was detected in 4 of 74 (5.4%) AD, PD, or AD + PD patients and 3 of 106 (2.8%) Caucasian controls [Shoffner et al., 1993]. The second variant is a nonconserved T to C transition at np 3197, which does not change an existing restriction endonuclease recognition site but has not been detected in at least 30 published mtDNA rRNA sequences [Brown et al., 1992a,b; Marzuki et al., 1991; Prezant et al., 1993; Wallace et al., 1988; Shoffner et al., 1990; Ozawa et al., 1991a,b; Kobayashi et al., 1990; Letrit et al., 1992]. Using primer-mismatch PCR and restriction endonucle-

TABLE I. MtDNA Mutations Found in ADPD and PD Patients*

Gene	Nucleotide position	Mutation	Amino acid change	Amino acid or nucleotide conservation H/C/M/X	Frequency in AD, PD, and ADPD patients (%)	Frequency in unaffected Caucasians (%)
Patient ADPD2[a]						
1. 16S rRNA	1721	C to T	—	C/C/C/T	4/74 (5.4)	3/106 (2.8)
2. 16S rRNA	3197	T to C	—	T/-/A/A	7/66 (10.6)	6/49 (12.2)
3. ND1	3397	A to G	M to V	M/M/M/M	2/173 (1.2)	0/248
4. ND2	4732	A to G	N to S	N/K/Q/D	2/74 (2.7)	1/106 (0.9)
5. ND5	13637	A to G	Q to R	Q/Q/K/H	4/74 (5.4)	3/106 (2.8)
Patient ADPD7[b]						
1. 12S rRNA	721	T to C	—	T/A/A/T	1/70 (1.4)	0/106
2. tRNA^{Gln}	4336	T to C	—	A/A/T/C	9/173 (5.2)	12/1691 (0.7)
3. ND5	13759	G to A	A to T	A/T/T/I	1/54 (1.9)	1/50 (2.0)
Patient ADPD14[c]						
1. ND1	4216	T to C	Y to H	Y/H/H/H	ND	13%
2. ND5	13708	G to A	A to T	A/L/A/A	6/71 (8.0)	6%
3. ND5	13934	C to T	T to M	T/M/I/I	2/62 (3.2)	0/107
Patient PD4[d]						
1. 12S rRNA	1189	T to C	—	T/T/T/C	7/73 (9.6)	6/48 (12.5)
2. 16S rRNA	1709	G to A	—	G/G/T/T	1/69 (1.5)	0/108
3. 16S rRNA	1811	A to G	—	A/A/T/A	14/71 (19.7)	9/48 (18.8)
4. Cytb	15851	A to G	I to V	I/A/S/M	1/66 (1.5)	0/108

* Mutations at np 3397 (ADPD2) and 4336 (ADPD7) have been described in detail by Shoffner et al. [1993]. References indicating polymorphism frequencies in various populations include: Howell et al. [1991a,b]; Marzuki et al. [1991]; Ballinger et al. [1992]; Brown et al. [1992a,b]; Torroni et al. [1992]; Shoffner et al. [1993, and unpublished data, this laboratory]. H, human; C, cow; M, mouse; X, *Xenopus* mtDNA.

[a] Patient APD2 also harbored the following common polymorphisms, Cambridge sequence errors, or synonymous (s) mutations at nps: 750, 1438, 2706, 3423, 4769, 6293 (s), 7028 (s), 7768 (s), 8860, 9477, 11335 (s), 11719, 13368 (s), 13617 (s), 13702, 14182 (s), 14199, 14272, 14365 (s), 14368, 15326, and 15721 (s).

[b] Patient ADPD7 also harbored the following common polymorphisms, Cambridge sequence errors, or synonymous (s) mutations: 750, 3423, 4769, 8860, 11335 (s), 13702, 14199, 14272, 14365 (s), 14368, 15326, and 15833 (s).

[c] Patient ADPD14 also harbored the following common polymorphisms, Cambridge sequence errors, or synonymous (s) mutations: 750, 2706, 3010, 3423, 4769, 7028 (s), 8860, 9428 (s), 10398, 11251 (s), 11335 (s), 12612 (s), 13702, 14199, 14365 (s), 14368, 14798, 15326. The Cambridge sequence error at np 14272 was not sequenced in this patient. ND, no data.

[d] Patient PD4 also harbored the following common polymorphisms, Cambridge sequence errors, or synonymous (s) mutations: 750, 1438, 2706, 3480 (s), 4769, 7028 (s), 8860, 9055, 10398, 10550 (s), 11229 (s), 11335 (s), 12308, 12372 (s), 13702, 14199, 14272, 14365, 14386, 14798, 15808 (s). The Cambridge sequence errors at np 3423 and 15326 were not sequenced in this patient.

ase digestion specific for this variant (see Materials and Methods), the np 3197 mutation was found in a total of 7 of 66 (10.6%) AD (n = 29), PD (n = 7), and AD + PD (n = 30) patients, with np 3197-positive patients having a similar mtDNA haplotype. Moreover, a total of 6 of 49 (12.2%) Caucasian controls harbored this variant, 3 with mtDNA haplotypes similar to the np 3197-positive patients and 3 with unknown haplotypes. Thus, this variant was not enriched in the patient population.

Patient ADPD14 harbored 21 mtDNA mutations compared to the Cambridge sequence (Table I). Eighteen of these mutations were excluded as silent mutations or errors in the Cambridge sequence. The 3 remaining variants all altered amino acids. The first was

Table II. Evolutionary Conservation of Amino Acids Altered by Rare Missense Mutations

Organism	ND2 mutation 4732 N to S Patient ADPD2	ND5 mutation 13637 Q to R Patient ADPD2	ND5 mutation 13759 A to T Patient ADPD7	Cytb mutation 13934 T to M Patient ADPD 14	ND5 mutation 15851 I to V Patient PD4
	*	*	*	*	*
Human	M T N T T	T G Q P R	S P A S P	S I T H R	P T I S L
Cow	V M K L F	L G Q P R	P P T T I	T I M H R	P T A G T
Mouse	F Q Q Q T	M T K P R	P P T S I	S I I H R	P I S G I
X. laevis	I L D L T	M G H P R	L P I N S	T I I H R	P L M G W
Sea urchin	I L D P V	S L S P S	F A - - P	D A V H L	P I V S S
Chicken	I T Q L N	T G H T R	L P P K T	P L T H R	P T I G T
Drosophila	N N E T N	T G D L N	N W L I F	Y I S T Y	P L V T K
C. elegans	- - - - -	- - - - -	W W M N F	- - - - -	F I F M S
A. suum	- - - - -	- - - - -	W W M N F	- - - - -	L V Y Y F

a T to C transition at np 4216 in the ND1 gene which changes a nonconserved tyrosine to a histidine and has been detected in 13% of unaffected controls [Johns and Berman, 1991; Brown et al., 1992a,b]. The second is a G to A transition at np 13708 in the ND5 gene which changes a moderately conserved alanine to a threonine and is present in 6% of the control population [Johns and Berman, 1991; Brown et al., 1992a,b]. Eight percent (6 of 71) of our AD and PD patients harbored this variant when screened using the diagnostic restriction endonuclease *Bst*NI. The np 4216 and 13708 mutations are often found on the same mtDNA haplotype and occur in an increased frequency in LHON patients, suggesting that at least one of these mutations (probably the 13708 mutation) may be a mildly pathogenic variant [Johns and Berman, 1991; Brown et al., 1992a,b]. The third mutation was a C to T transition at np 13934 in the ND5 gene. This mutation changes a nonconserved threonine to a methionine and has been detected in the mtDNA of a single LHON patient [Howell et al., 1991a], although no pathogenic role was suggested for this mutation. Using primer-mismatch PCR and restriction endonuclease digestion specific for this variant, the np 13934 mutation was found in a total of 2 of 62 (3.2%; including patient ADPD 14) AD (n = 30), PD (n = 8), and AD + PD (n = 24) patients. The 2 np 13934-positive patients had different mtDNA haplotypes. This mutation was not found in 107 Caucasian or 40 African controls, but was found in one of 40 (2.5%) Asian mtDNAs screened. Thus, this mutation has occurred at least 2 independent times in human evolution and, in Caucasians, has only been found in patients. However, the low number of positive patients makes it difficult to assign a pathogenic role to this variant.

Patient PD4 harbored 25 mtDNA mutations compared to the Cambridge sequence. Twenty-one of these were excluded as above, leaving one missense mutation and 3 rRNA mutations. The missense variant, an A to G transition at np 15851 of the cytochrome b gene, changes a nonconserved isoleucine to a valine. This variant was found in one of 7 patients with hypertrophic cardiomyopathy [Obayashi et al., 1992], but a pathogenic role was not ascribed to it. The np 15851 mutation creates a *Bsm*AI restriction endonuclease site and was therefore detected in a total of one of 66 (1.5%) AD (n = 29), PD (n = 7), and AD + PD (n = 30) patients, PD4 being the only positive patient. This variant was not detected in 172 controls including 108 Caucasians, 40 Asians, and 24 African mtDNAs. The 3 rRNA polymorphisms change nonconserved rRNA nucleotides. Primer-mismatch PCR and restriction enzyme digestion assays specific for the T to C transition at np 1189 in the 12S rRNA gene and the A to G transition at np 1811 in the 16S rRNA gene revealed that the np 1189 variant was found in 7 of 73 (9.6%) AD and PD patients and in 6 of 48 (12.5%) Caucasian controls, while the np 1811 variant was found in 14 of 71 (19.7%) AD and PD patients and in 9 of 48 (18.8%) Caucasian controls, indicating that these are neutral polymorphisms. The np 1811 polymorphism has also been observed in a single myoclonic epilepsy and ragged red fiber (MERRF) disease patient [Marzuki et al., 1991]. The third rRNA variant, a G to A transition at np 1709 of the 16S rRNA gene, was also analyzed using primer-mismatch PCR and mutation-specific restriction digestion. Of 69 patients (31 AD, 7 PD, and 31 AD + PD patients) tested, only patient PD4 was positive, giving an overall frequency of one of 69 (1.5%). This variant was not detected in 108 Caucasian and 40 African controls, but was found in 2 of 40 (5.0%) Asian mtDNAs. Thus, this mutation has occurred at least twice in human evolution, but its low frequency in patients makes it impossible to assign an etiological role to this variant.

DISCUSSION

To fully investigate the role of mtDNA point mutations in AD, PD, and AD + PD, we sequenced the mitochondrial rRNA, tRNA, and protein-coding genes from 3 AD + PD patients and one PD patient. The results of this analysis indicate that a common pathogenic mtDNA mutation was not shared by the patients studied and that the np 4336 variant (patient ADPD7) in $tRNA^{Gln}$ and perhaps the np 3397 variant (patient ADPD2) in ND1 are the mtDNA mutations in these patients which have characteristics of pathological mtDNA mutations and thus may have a causal role. Likewise, and by extrapolation from LHON, the np 4216 variant in ND1 and the np 13708 variant in ND5 may contribute to the pathology in ADPD14. The functional significance of the mtDNA variants in patient PD4 is unclear.

For the ADPD7 mtDNA, the np 4336 $tRNA^{Gln}$ variant was previously shown to be at increased frequency in AD and PD patients [Shoffner et al., 1993]. It alters a moderately conserved tRNA nucleotide, occurs 7.4 times more frequently in patients than in a large number of controls, and defines an mtDNA lineage where the prevalence of AD, PD, and AD + PD is high. In a separate and more recent case-control study, Hutchin and Cortopassi [1995] reported this mutation to be mtDNA lineage specific and to be 24 times more common in their cohort of 72 AD cases than in 296 race- and age-matched controls. In addition to the np 4336 variant, this patient's mtDNA also contained an np 721 variant in the 12S rRNA gene which is unique to the patient. Thus, it appears that the np 4336 mutation could have a causal role in these neurodegenerative diseases and is not simply a "hitchhiking" marker linked to another, more significant mtDNA mutation. Since 2 other ADPD patients on the $tRNA^{Gln4336}$ lineage have each been observed to harbor an additional potentially pathogenic mutation [at np 3397 in ND1 and the 5 nucleotide insertions at np 956-965 in the 12S rRNA gene; Shoffner et al., 1993], it remains possible that the np 721 variant interacts with the np 4336 mutation to increase the probability of disease expression, analogous to the situation postulated for LHON [Johns and Neufeld, 1991; Brown et al., 1992a,b; Johns et al., 1992].

For the ADPD2 mtDNA, the only potentially pathogenic mutation found was the np 3397 variant in the ND1 gene. This transition alters an evolutionarily conserved amino acid, was not found in 248 Caucasian controls [Shoffner et al., 1993], and changes the amino acid adjacent to a highly conserved tyrosine altered in some LHON patients [Brown et al., 1992a; Johns et al., 1992]. No other potentially pathologic variants were

found in this mtDNA, in contrast to the other np 3397-positive patient [ADPD1 in Shoffner et al., 1993], which harbors the np 4336 variant.

The mtDNA variants found in patients ADPD14 and PD4 are of particularly uncertain pathogenicity. For patient ADPD14, the np 13708 mutation and perhaps the np 13934 mutation may influence disease expression. The 13708 ND5 mutation alters a moderately conserved amino acid and is present at an increased frequency in LHON patients. The accompanying 13934 ND5 mutation may be an interactive factor since it was found in 2 patients, but no Caucasian controls. Therefore, it is possible that these mutations subtly inhibit complex I and interact synergistically with other genetic and/or environmental factors to cause the disease, however it is also possible that these mutations have no etiological role in these late-onset diseases.

Patient PD4 harbored 2 rare mutations at np 15851 and np 1709 that were not found in over 100 Caucasian controls. The np 15851 variant was also not found in 40 Asian or 24 African mtDNAs, but was detected in a hypertrophic cardiomyopathy patient [Obayashi et al., 1992]. The np 1709 variant was found in 2 Asian and no African mtDNAs. Thus, these 2 mutations remain candidates for causal mutations although it is presently equally probable that they are rare neutral polymorphisms.

In conclusion, the detailed mtDNA sequence analysis of ADPD7 and ADPD2 supports the hypothesis that the np 4336 $tRNA^{Gln}$ variant and perhaps the np 3397 ND1 variant are the mtDNA mutations most likely to contribute to AD and/or PD pathology, with the np 721 12S rRNA variant in ADPD7 also possibly contributing to these diseases. Analysis of the ADPD14 and PD4 mtDNAs indicates that the np 13708 ND5 and np 13934 variants in ADPD14 and the np 1709 16S rRNA and np 15851 cytochrome b variants in PD4 merit further investigation. However, considerably more supportive data will be required before these or any mtDNA mutations can be unequivocally ascribed a pathological role in AD and PD.

ACKNOWLEDGMENTS

The authors thank Dr. Antonio Torroni and Ms. Marie Lott for their contributions to this project. This study was supported by National Institutes of Health grants AG101309 and NS21328.

REFERENCES

Anderson S, Bankier A, Barrell BG, deBruijin MHL, Coulson AR, Drouin J, Eperon JC, Nierlich DP, Rose BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981): Sequence and organization of the human mitochondrial genome. Nature 290: 457–465.

Ballinger SW, Schurr TG, Torroni A, Gan YY, Hodge JA, Hassan K, Chen KH, Wallace DC (1992): Southeast Asian mitochondrial DNA analysis reveals genetic continuity of ancient mongoloid migrations. Genetics 130:139–152.

Benecke R, Stumper P, Weiss H (1993): Electron transfer complexes I and IV of platelets are abnormal in Parkinson's disease but normal in Parkinson-plus syndromes. Brain 116:1451–1463.

Bindoff L, Birch-Machin MA, Cartlidge NEF, Parker WD Jr, Turnbull DM (1991): Respiratory chain abnormalities in skeletal muscle from patients with Parkinson's disease. J Neurol Sci 104:203–208.

Blin O, Desnuelle C, Rascol O, Borg M, Peyro Saint Paul H, Azulay JP, Bille F, Figarella D, Coulom F, Pellissier JF, Montastruc JL, Chatel M, Serratrice G (1994): Mitochondrial respiratory failure in skeletal muscle from patients with Parkinson's disease and multiple system atrophy. J Neurol Sci 125:95–101.

Boffoli D, Scacco SC, Vergari R, Solarino G, Santacroce G, Papa S (1994): Decline with age of the respiratory chain activity in human skeletal muscle. Biochem Biophys Acta 1226:73–82.

Bowling AC, Mutisya EM, Walker LC, Price DL, Cork LC, Beal MF (1993): Age-dependent impairment of mitochondrial function in primate brain. J Neurochem 60:1964–1967.

Brown MD, Voljavec AS, Lott MT, MacDonald I, Wallace DC (1992a): Leber's hereditary optic neuropathy: a model for mitochondrial neurodegenerative disease. FASEB J 6:2791–2799.

Brown MD, Voljavec AS, Lott MT, Torroni A, Yang C, Wallace DC (1992b): Mitochondrial DNA complex I and III mutations associated with Leber's hereditary optic neuropathy. Genetics 130: 163–173.

Byrne EJ, Lennox G, Lowe J, Godwin-Austin R (1989): Diffuse Lewy body disease: Clinical features in 15 cases. J Neurol Neurosurg Psychiatry 52:709–717.

Cardellach F, Marti MJ, Fernandez-Sola J, Marin C, Hoek JB, Tolosa E, Urbano-Marquez A (1993): Mitochondrial respiratory chain activity in skeletal muscle from patients with Parkinson's disease. Neurology 43:2258–2262.

Chui HC, Teng EL, Henderson VW, Moy AC (1985): Clinical subtypes of dementia of the Alzheimer type. Neurology 35:1544–1550.

Cooper JM, Mann VM, Schapira AHV (1992): Analyses of mitochondrial respiratory chain function and mitochondrial DNA deletion in human skeletal muscle: Effect of aging. J Neurol Sci 113:91–98.

Corral-Debrinski M, Stepien G, Shoffner JM, Lott MT, Kanter K, Wallace DC (1991): Hypoxia is associated with mitochondrial DNA damage and gene induction. Implications for cardiac disease. JAMA 266:1812–1816.

Crain BJ, Mirra SS (1990): Degenerative diseases. Curr Opinion Neurol Neurosurg 921–925.

Dickson DW, Crystal H, Mattiace LA, Kreess Y, Schwagrl A, Dsiezak-Reding H, Davies P, Yuen S-HC (1989): Diffuse Lewy body disease: Light and electron microscopic immunocytochemistry of senile plaques. Acta Neuropathol 78:572–584.

Ditter SM, Mirra SS (1987): Neuropathologic and clinical features of Parkinson's disease in Alzheimer's disease patients. Neurology 37:754–760.

Golbe LI (1990): The genetics of Parkinson's disease: A reconsideration. Neurology 40(suppl 3):7–14.

Hansen L, Salmon D, Galasko D, Masliah E, Katzman R, DeTeresa R, Thal L, Pay MM, Hofstetter R, Klauber M, Rice V, Butters N, Alford M (1990): The Lewy body variant of Alzheimer's disease: A clinical and pathologic entity. Neurology 40:1–8.

Hansen LA, Masliah E, Terry RD, Mirra SS (1989): A neuropathological subset of Alzheimer's disease with concomitant Lewy body disease and spongiform change. Acta Neuropathol 78:194–201.

Hattori N, Tanaka M, Ozawa T, Mizuno Y (1991): Immunohistochemical studies on complexes I, II, III, and IV of mitochondria in Parkinson's disease. Ann Neurol 30:563–571.

Howell N, Bindoff LA, McCullough DA, Kubacka I, Poulton J, Mackey D, Taylor L, Turnbull DM (1991a): Leber hereditary optic neuropathy: Identification of the same mitochondrial ND1 mutation in six pedigrees. Am J Hum Genet 49:939–950.

Howell N, Kubacka I, Halvorson S, Mackey D (1993): Leber's hereditary optic neuropathy: The etiological role of a mutation in the mitochondrial cytochrome b gene. Genetics 133:133–136.

Howell N, Kubacka I, Xu M, McCullough DA (1991b): Leber's hereditary optic neuropathy: Involvement of the mitochondrial ND1 gene and evidence for an intragenic suppressor mutation. Am J Hum Genet 48:935–942.

Hutchin T, Cortopassi G (1995): A mitochondrial DNA clone is associated with increased risk for Alzheimer disease. Proc Natl Acad Sci USA 92:6892–6895.

Johns DR, Berman J (1991): Alternative, simultaneous complex I mitochondrial DNA mutations in Leber's hereditary optic neuropathy. Biochem Biophys Res Commun 174:1324–1330.

Johns DR, Neufeld MJ (1991): Cytochrome b mutations in Leber's hereditary optic neuropathy. Biochem Biophys Res Commun 181:1358–1364.

Johns DR, Neufeld MJ, Park RD (1992): An ND-6 mitochondrial DNA mutation associated with Leber hereditary optic neuropathy. Biochem Biophys Res Commun 187:1551–1557.

Johnson WB (1991): Genetic susceptibility in Parkinson's disease. Neurology 41:82–87.

Kobayashi Y, Monoi MY, Tominga K, Momoi T, Nihei K, Yanagisawa M, Kagawa Y, Ohta S (1990): A point mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene in MELAS (mitochondrial myopathy, encephalomyopathy, lactic acidosis, and stroke-like episodes). Biochem Biophys Res Commun 173:816–822.

Krige D, Carroll MT, Cooper JM, Marsden D, Shapira AHV (1992): Platelet mitochondrial function in Parkinson's disease. Ann Neurol 32:782–788.

Letrit P, Noer AS, Jean-Francois MJ, Kapsa R, Dennett X, Thyagarajan D, Lethkan K, Byrne E, Marzuki S (1992): A new disease-related mutation for mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS) syndrome affects the ND4 subunit of respiratory complex I. Am J Hum Genet 51:457–468.

Mackey D, Howell N (1992): A variant of Leber hereditary optic neuropathy characterized by recovery of vision and by an unusual mitochondrial genetic etiology. Am J Hum Genet 51:1218–1228.

Mann VM, Cooper JM, Krige D, Daniel SE, Shapira AHV, Marsden CD (1992): Brain, skeletal muscle, and platelet mitochondrial function in Parkinson's disease. Brain 115:333–342.

Marzuki S, Noer AS, Letrit P, Thyagarajan D, Kapsa R, Utthanaphol P, Byrne E (1991): Normal variants of human mitochondrial DNA and translation products: The building of a reference data base. Hum Genet 88:139–145.

Mayeux R, Stern Y, Spanton S (1985): Heterogeneity in dementia of the Alzheimer type: Evidence of subgroups. Neurology 35: 435–461.

Mizuno Y, Ohta S, Tanaka M, Takamiya S, Suzuki K, Sato T, Oya H, Ozawa T, Kagawa Y (1989): Deficiencies in complex I subunits of the respiratory chain in Parkinson's disease. Biochem Biophys Res Commun 163:1450–1455.

Mullan M, Houlden H, Windelspecht M, Fidani L, Lombardi C, Diaz P, Rossor M, Crook R, Hardy J, Duff K, Crawford F (1992): A locus for familial early-onset Alzheimer's disease on the long arm of chromosome 14, proximal to the 1-antichymotrypsin gene. Nature Genet 2:340–342.

Nakagawa-Hattori Y, Yoshino H, Kondo T, Mizuno Y, Horai S (1992): Is Parkinson's disease a mitochondrial disorder? J Neurol Sci 107: 29–33.

Obayashi T, Hattori K, Sugiyama S, Tanaka M, Tanaka T, Itoyama S, Deguchi H, Kawamura K, Koga Y, Toshima H, Takeda N, Nagano M, Ito T, Ozawa T (1992): Point mutations in mitochondrial DNA in patients with hypertrophic cardiomyopathy. Am Heart J 124: 1263–1269.

Ozawa T, Tanaka M, Ino H, Ohno K, Sano T, Wada Y, Yoneda M, Tanno Y, Miyatake T, Tanaka T, Itoyama S, Ikebe S, Hattori N, Mizuno Y (1991a): Distinct clustering of point mutations in mitochondrial DNA among patients with mitochondrial encephalomyopathies and with Parkinson's disease. Biochem Biophys Res Commun 176:938–946.

Ozawa T, Tanaka M, Sugiyama S, Ino H, Ohno K, Hattori K, Ohbayashi T, Ito T, Deguchi H, Kawamura K, Nakane Y, Hashiba K (1991b): Patients with idiopathic cardiomyopathy belong to the same mitochondrial DNA gene family of Parkinson's disease and mitochondrial encephalomyopathy. Biochem Biophys Res Commun 177:518–525.

Parker WD, Boyson SJ, Parks JK (1989): Abnormalities of the electron transport chain in idiopathic Parkinson's disease. Ann Neurol 26:719–723.

Parker WD, Filley CM, Parks JK (1990): Cytochrome c oxidase deficiency in Alzheimer's disease. Neurology 40:1302–1303.

Parker WD, Mahr NJ, Filley CM, Parks JK, Hughes D, Young DA, Cullum CM (1994a): Reduced platelet cytochrome c oxidase activity in Alzheimer's disease. Neurology 44:1086–1089.

Parker WD, Parks JK, Filley CM, Kleinschmidt-DeMasters BK (1994b): Electron transport chain defects in Alzheimer's disease brains. Neurology 44:1909–1096.

Perry RH, Irving D, Blessed Fairbairn A, Perry EK (1990): Senile dementia of the Lewy body type: A clinically and neuropathologically distinct form of Lewy body dementia in the elderly. J Neurol Sci 95:119–139.

Peterson C, Goldman JE (1986): Alterations in calcium content and biochemical processes in cultured skin fibroblasts from aged and Alzheimer's donors. Proc Natl Acad Sci USA 83:2758–2762.

Prezant TR, Agapian JV, Bohlman MCB, Bu X, Oztas S, Qi W-Q, Arnos KS, Cortopassi GA, Jaber L, Rotter JI, Shohat M, Fischel-Ghodsian N (1993): Mitochondrial ribosomal RNA mutation associated with both antibiotic-induced and non-syndromic deafness. Nature Genet 4:289–294.

Saraiva AA, Borges MM, Madeira MD, Tavares MA, Paula-Barbosa MM (1985): Mitochondrial abnormalities in cortical dendrites from patients with Alzheimer's disease. J Submicrosc Cytol 17:459–464.

Schapira AHV (1994): Evidence for mitochondrial dysfunction in Parkinson's disease—A critical appraisal. Mov Disord 9:125–138.

Schapira AH, Cooper JM, Dexter D, Clark JB, Jenner P, Marsden CD (1990): Mitochondrial complex I deficiency in Parkinson's disease. J Neurochem 54:823–827.

Schapira AH, Cooper JM, Dexter D, Jenner P, Clark JB, Marsden CD (1989): Mitochondrial complex I deficiency in Parkinson's disease. Lancet 1:1269.

Shoffner JM, Brown MD, Torroni A, Lott MT, Cabell MF, Mirra SS, Beal MF, Yang CC, Gearing M, Salvo R, Watts RL, Juncos JL, Hansen LA, Crain BJ, Fayad M, Reckord CL, Wallace DC (1993): Mitochondrial DNA variants observed in Alzheimer disease and Parkinson disease patients. Genomics 17:171–184.

Shoffner JM, Lott MT, Lezza AM, Seibel P, Ballinger SW, Wallace DC (1990): Myoclonic epilepsy and ragged-red fiber disease (MERRF) is associated with a mitochondrial DNA tRNA(Lys) mutation. Cell 61:931–937.

Shoffner JM, Watts RL, Juncos JL, Torroni A, Wallace DC (1919): Mitochondrial oxidative phosphorylation defects in Parkinson Disease. Ann Neurol 30:332–339.

St. George-Hyslop P, Haines J, Rogaev E, Mortilla M, Vala G, Pericak-Vance M, Foncin J-F, Montesi M, Bruni A, Sorbi S, Rainero I, Pinessi L, Pollen D, Polinski R, Nee L, Kennedy J, Macciardi F, Rogaeva E, Liang Y, Alexandrova N, Lukiw W, Schlumpf K, Tanzi R, Tsuda T, Farrer L, Cantu J-M, Duara R, Amaducci L, Bergamini L, Gusella J, Roses A, Crapper-McLachlan D (1992): Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. Nature Genet 2:330–334.

Strittmatter WJ, Saunders A, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD (1993): Apolipoprotein E: High-avidity binding to β-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 90:1977–1981.

Torroni A, Miller JA, Moore LG, Zamudio S, Zhuang J, Droma T, Wallace DC (1994): Mitochondrial DNA analysis in Tibet. Implications for the origin of the Tibetan population and its adaptation to high altitude. Am J Phys Anthropol 93:189–199.

Torroni A, Schurr T, Yang C-C, Szathmary EJE, Williams RC, Schanfield MS, Troup GA, Knowler WC, Lawrence DN, Weiss KM, Wallace DC (1992): Native American mitochondrial DNA analysis indicates that the Amerind and the Nadene populations were founded by two independent migrations. Genetics 130:153–162.

Torroni A, Schurr TG, Cabell MF, Brown MD, Neel JV, Larsen M, Smith DG, Vullo CM, Wallace DC (1993a): Asian affinities and continental radiation of the four founding Native American mitochondrial DNAs. Am J Hum Genet 53:563–590.

Torroni A, Sukernik RI, Schurr TG, Starikovskaya YB, Cabell MF, Crawford MH, Comuzzie AE, Wallace DC (1993b): Mitochondrial DNA variation of Aborginal Siberians reveals distinct genetic affinities with Native Americans. Am J Hum Genet 53:591–608.

Trounce I, Byrne E, Marzuki S (1989): Decline in skeletal muscle mitochondrial respiratory chain function: Possible factor in aging. Lancet 1:637–639.

Van Broeckhoven C, Backhovens H, Cruts M, De Winter G, Bruyland M, Cras P, Martin J-J (1992): Mapping of a gene predisposing to early-onset Alzheimer's disease to chromosome 14q24.3. Nature Genet 2:335–339.

Wallace DC (1992a): Diseases of the mitochondrial DNA. Annu Rev Biochem 61:1175–1212.

Wallace DC (1992b): Mitochondrial genetics: A paradigm for aging and degenerative diseases? Science 256:628–632.

Wallace DC, Singh G, Lott MT, Hodge JA, Schurr TG, Lezza AMS, Elsas LJ, Nikoskelainen EK (1988): Mitochondrial DNA mutation associated with Leber's hereditary optic neuropathy. Science 289 242:1427–1430.

Yoshino H, Nakagawa-Hattori Y, Knodo T, Mizuno Y (1991): Mitochondrial complex I and II activities of lymphocytes and platelets in Parkinson's disease. J Neurol Transm 4:27–34.